



Figure 1A

SEQ ID NO:1

/translation="MGSVLSTDSGKSAPASATARALERRRDPELPVTSFDCAVCLEVL
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CDTLVCLSEMRAHIRT CQKYIDKYGPLQELEETAARCVCPFCQRELYEDSLLDHCITH
HRSERRPVFCPLCRLIPDENPSSFSGNLIRHLQVSHTLFYDDFIDFNIIEEALIRRVL
DRSLLEYVNHSNTT"

Figure 1B

SEQ ID NO:2

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LEWIS & CLARK, 1804-1806, in *THE JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION*

Mouse TRAC1 cDNA sequence:

35

Mouse TRAC1 protein (3rd frame)

SAXXGSLSSDSSKSAPASATPRTLERSGDSELPITSFDCSVCLEVLHQPV
VRTRCGHVFCRSCIATSIKNNKWTCPYCRAYLPSEGV PATDIAKRMKSE
YQNAECGTLVCLSDMRAHIRTCEKYIDKYGPLLELGDTTARCVCPFCQR
ELDEDCLLDHCI IHRSERRPVFCPLCHSRPDESPSTFNGSLIRHLQVSH
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FIGURE 2A

FLJ20456 Hit Inhibited anti-TCR Induced CD69 Expression in Jurkat Cells



Original clone

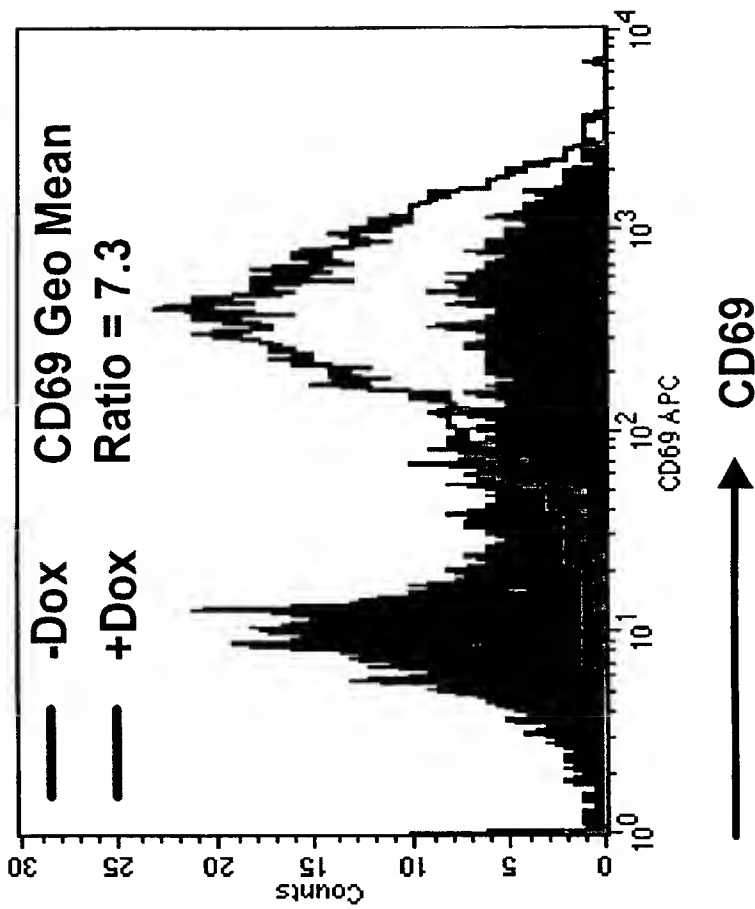


FIGURE 2B
FLJ20456 Hit Inhibited anti-TCR Induced
CD69 Expression in Jurkat Cells
Phenotype Transfer

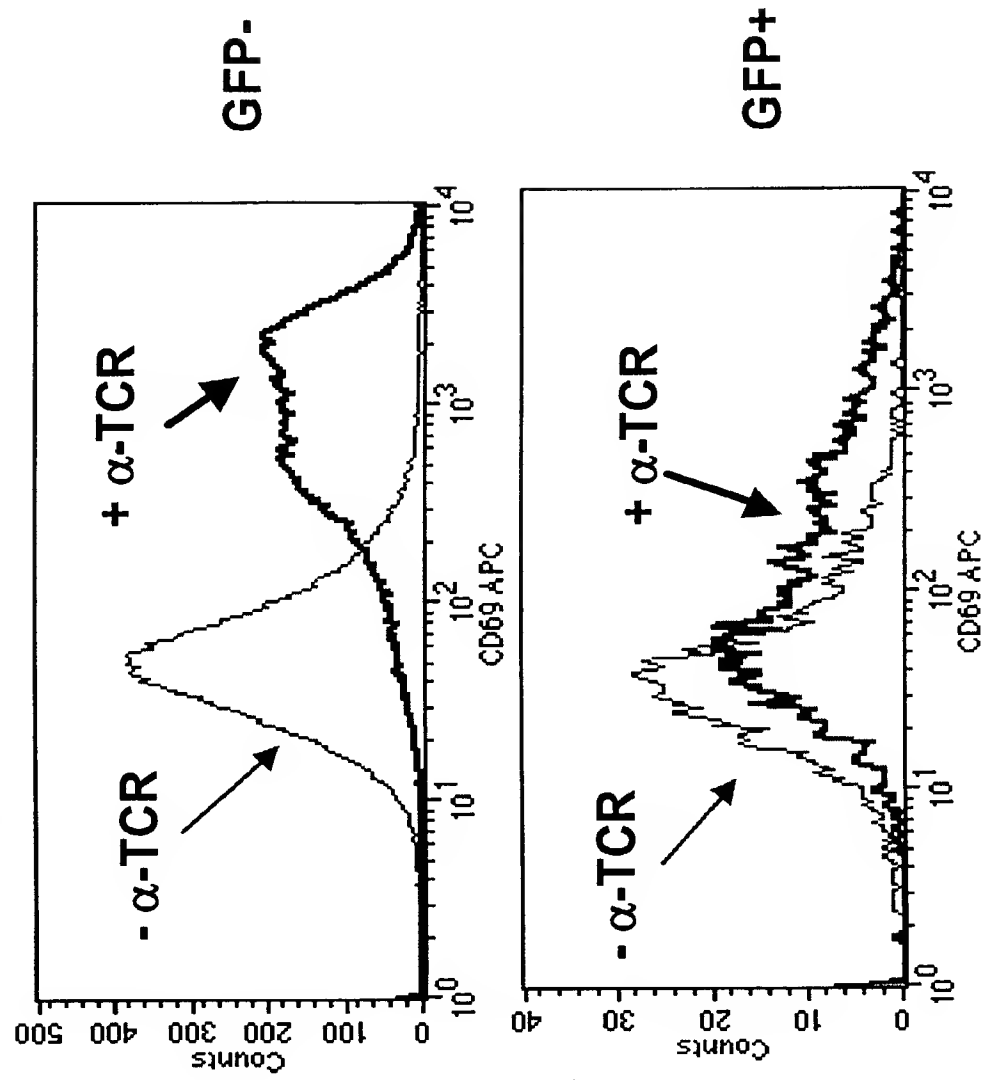


Figure 3A



- # JurkatN 32H

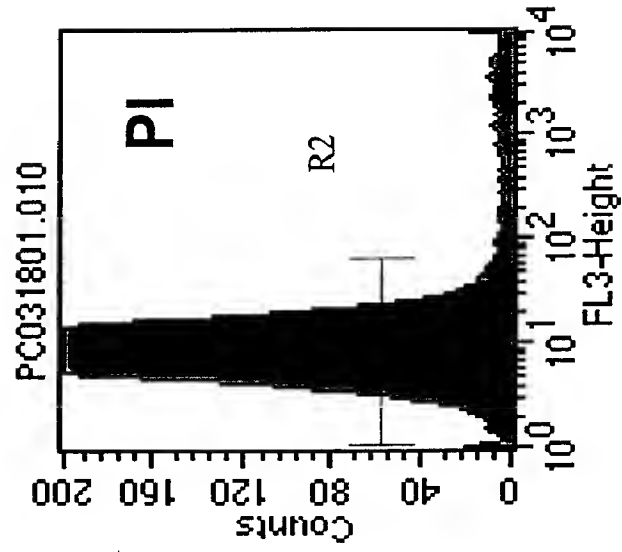
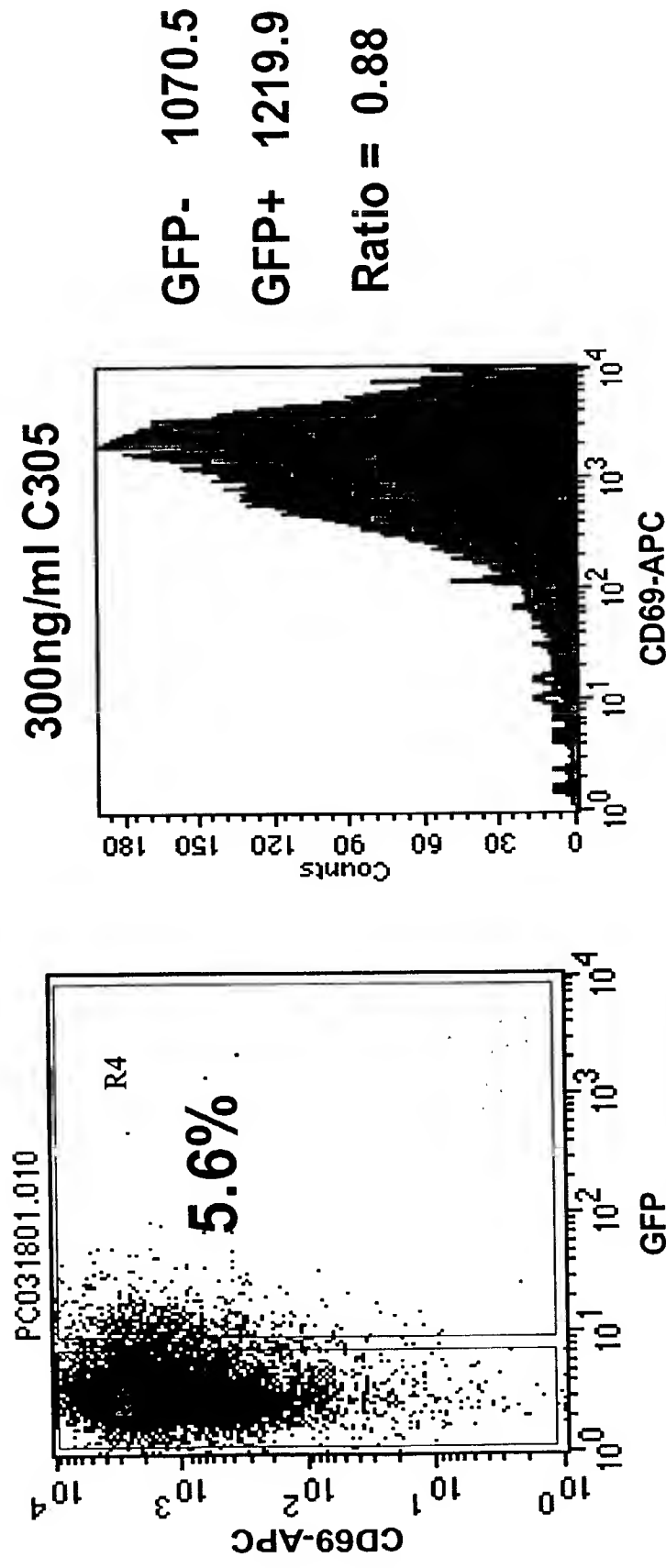


Figure 3B

Full Length FLJ20456 Does Not Inhibit CD69 Upregulation in Jurkat Cells



GFP- 1070.5

GFP+ 1219.9

Ratio = 0.88

Figure 4A

FLJ20456 Hit Specifically Inhibited T Cell Activation but not B Cell Activation

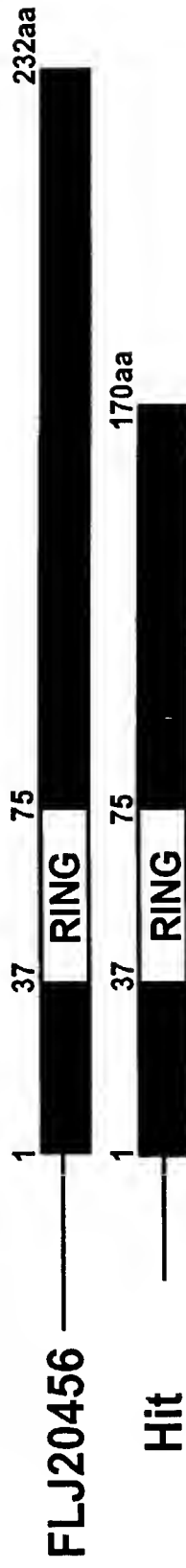


Figure 4B

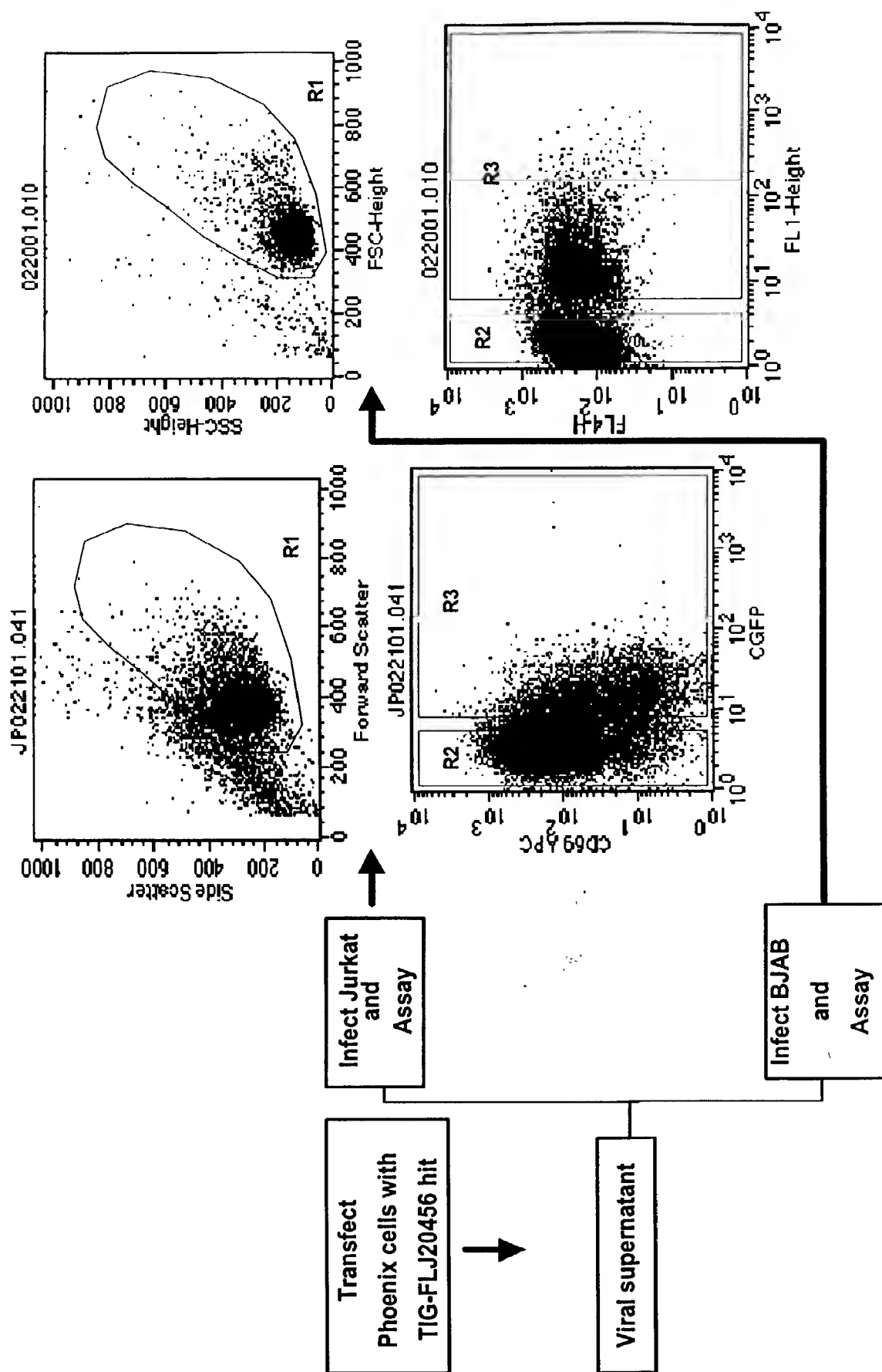
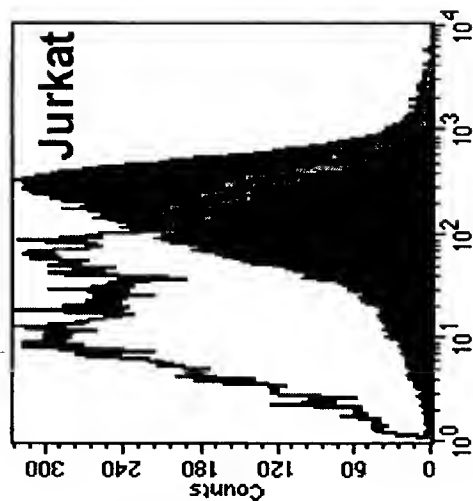


Figure 4C



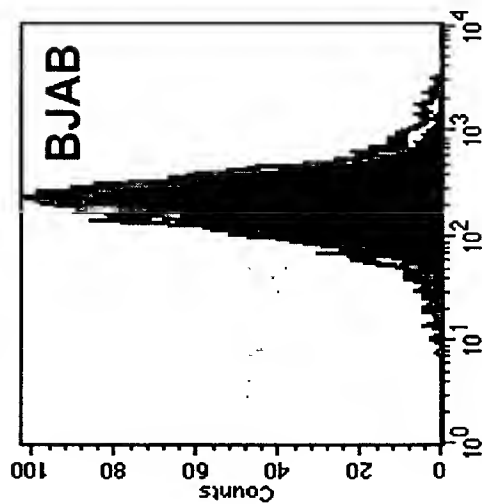
CD69-APC

Stimulation:
300ng/ml C305

GFP- 141.2

GFP+ 28.8

Ratio = 4.9



CD69-APC

Stimulation:
300ng/ml α -IgM

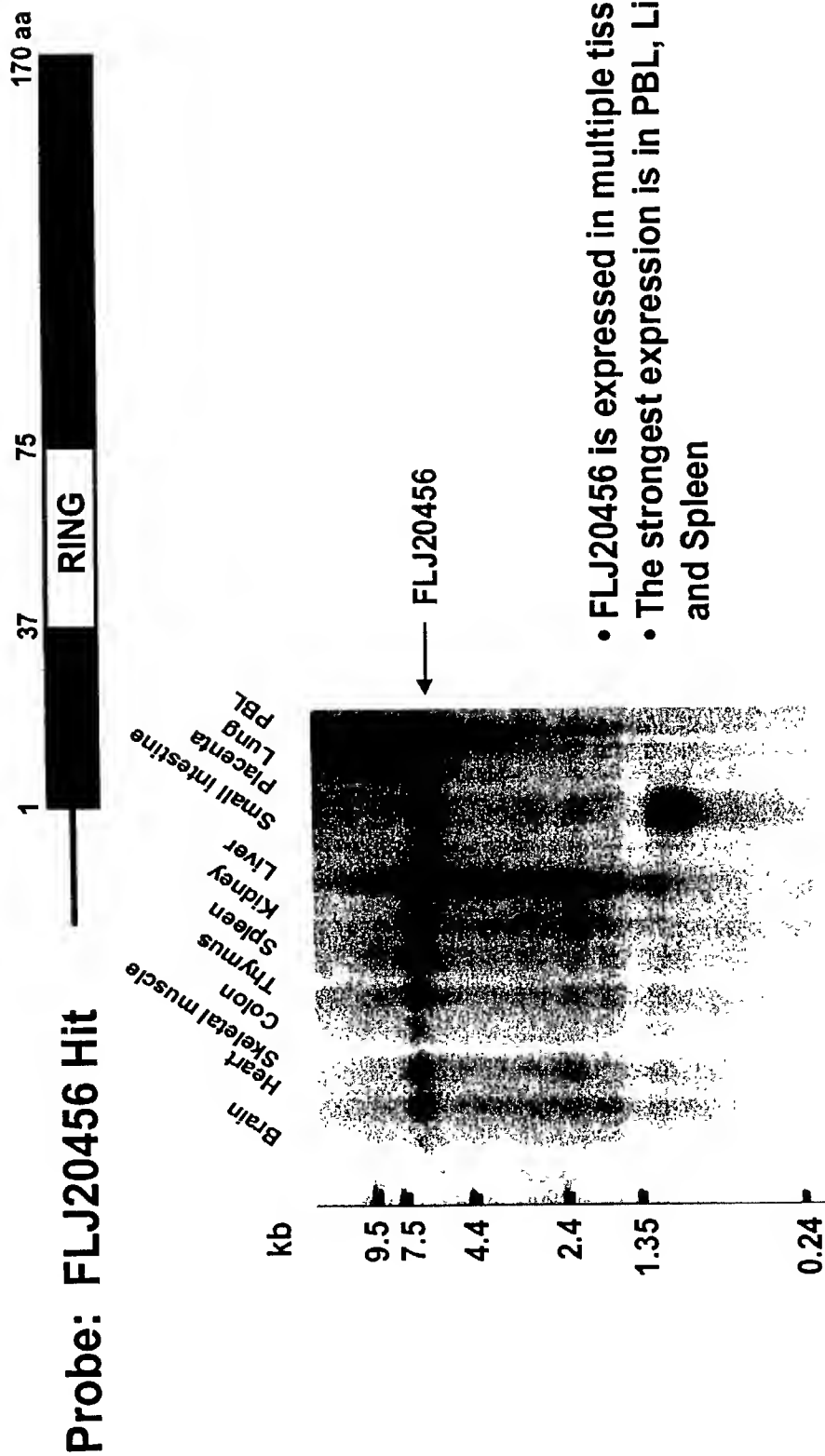
GFP- 183.1

GFP+ 183.3

Ratio = 1.0

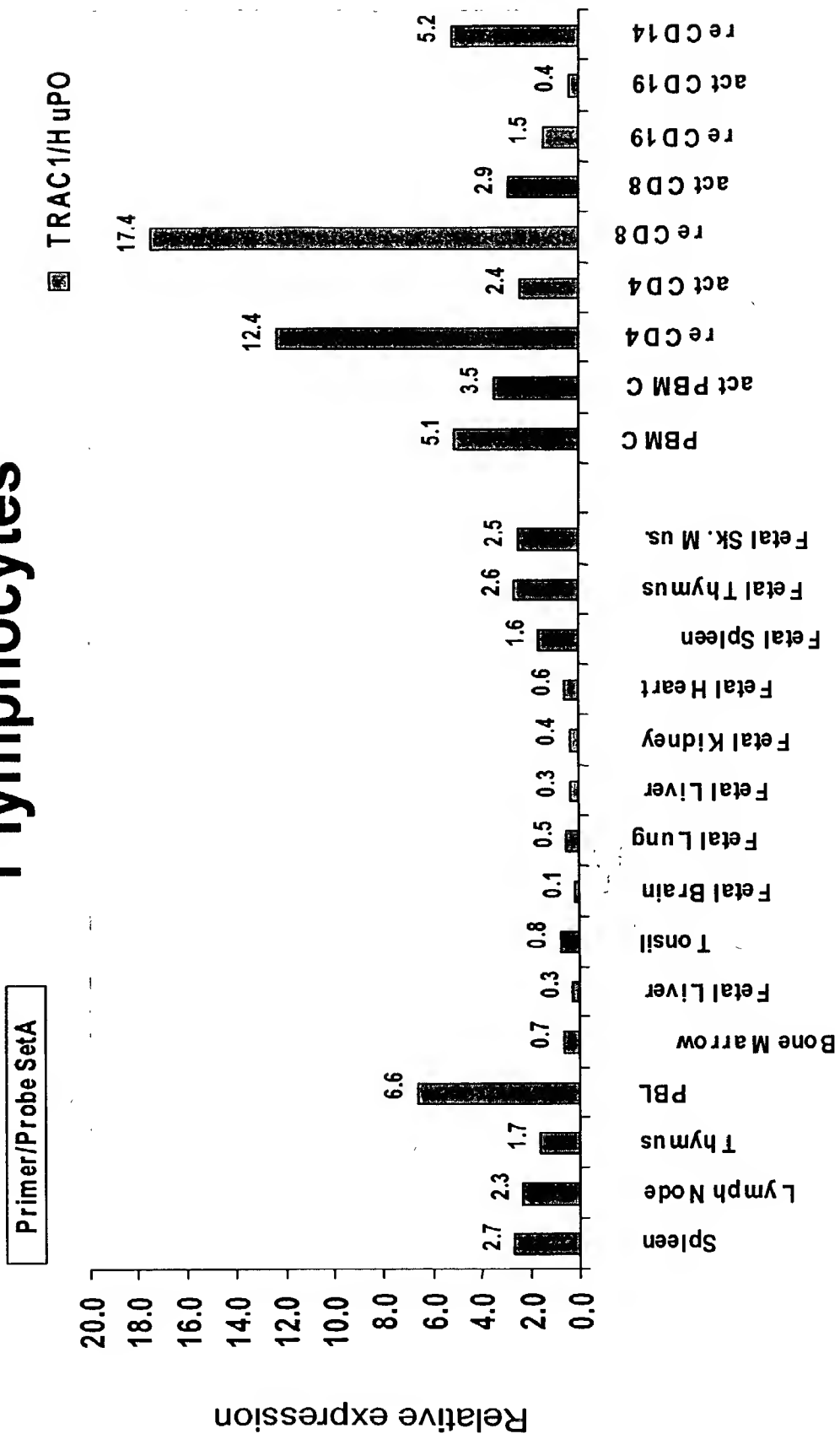
Figure 5

FLJ20456 Is Strongly Expressed in Lymphoid and Hematopoietic Organs



TRAC1 is Predominantly Expressed in Resting T lymphocytes

Figure 6



FL20456 Sequence is Most Similar to Two Sequences: Znf313 and STRIN

Ring Domain

Consensus #1F.C.VC.EV...PV....C.HVFC..C.....

FLJ20456.ppep MGSVLSTDSGKSAPASATAPATERRRRDPELPTVSTFCVAVCLEVLHQPVPTTRCGHVFCRSCIAITSLKN 67

znf313.ppep -----MAAQQRDCGGAQIAGFAAEADPLGRFTCPVCLVEYEKPVQV-PCGHVFCSAQLQECLKP 59

STRIN.ppep -----MAEDLSAATSYTEDEDEYCEVQCEVLKTPVPTTACQHVFCRKCFLTAMRE 49

Consensus #1C..CR.....A.....C.C.....R.H...C.KY...Y.....

FLJ20456.ppep NRTCEYCRAYTPSE-----GVPTDVAKRMKSEYKNCAECDTLVCLSEMRARIHTCCKYIDKYGPLQE 131

znf313.ppep KKPVCGVCRSALAP-----GVRVLELEROIESTETSTCHGCRNFFLSKIRSHVATCSKYQN-YIMEGV 121

STRIN.ppep SGACHPLCRGNVTRRERACPERALDENIMRKFSGSCRCCKAKQIKFYRMTHYKSCCKYQDEYGVSSI 117

Consensus #1CP.C.....L..HC...H.....

FLJ20456.ppep LEETAAR-----CVCFFQRELYE-DSLDDHCITHRSERR 166

znf313.ppep KATIKDASLQPRNVPNRY-----TFPCPYCEPKNFDDQGLVEHCKLHFSTDTK 169

STRIN.ppep VPNFQISQDSVGNRSETSTSDNTETTYQENTSSSGHPTEKCPLEQESNFTRQRLDDHCNSNHLFQIV 185

Consensus #1 .V.CP.C...P...P....N...H.....Y..F.....EE.....S.....-

FLJ20456.ppep PMFCPLCRLIPDENPSSFSGNLIRHLQVSHITLFYDDFLDFNIEEALIRRVLDRLSLLLEVNHSTNT. 233

znf313.ppep SMVCPICASMPWGDPNYRSANFREHTQRRHRESYDTFVDYDVEEDMMNQVLORSIIDQ. 229

STRIN.ppep PMTCPLCVSLPWGDESQITRNFSHLNORRQEDYGEEMNLQIDEETQYQTAVEESFQVNI. 246

* All three sequences are human
 * Murine sequences are not shown

	1	2	3	
1		26.6	22.3	1
2	130.4		27.9	2
3	140.9	134.7		3
	1	2	3	
				FLJ20456.ppep
				znf313.ppep
				STRIN.ppep

FIG. 7.

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Alignment of RING Domain Sequences from Various Human Proteins

Consensus #1C..C.....C.H..C.C.....C.....C.....

FLJ20456.Ring	VTSFDCAVCL	EV	LHQ	PVR	TR	-CGHVF	RSC	IAT	SL--KNNK	WT	CP	YCR	AYLP-S	50
znf313.Ring	LGRFTCPVCL	EV	YEK	PVQ	VP	-CGHVF	C	SAC	LQ	ECL--KPKK	PV	CGV	CRSALA-P	50
STRIN.Ring	EDDFYCPV	Q	EV	LKT	PVRT	TAC	Q	HVF	CR	KCF	L	TAM--RESGA	HCP	50
TRAF6.Ring	ESKYECPI	CL	MA	L	REAV	Q	TP	-CGHRE	CK	AC	I	IKSI--RDAGH	KCP	50
c-Cbl.Ring	STFQLCKI	CA	EN	-DKD	V	KIE	P	CGHLM	CT	S	CL	TSW--QESE	GQ	50
BRCA1.Ring	---LEC	PIC	LE	L	I	KE	P	V	STK-CD	HI	F	CK	F	50
BAR.Ring	VSEFSCH	CC	YD	I	L	V	N	P	T-TL	NC	G	H	S	50
RAG1.Ring	VKSIS	Q	I	C	E	H	I	L	A	D	P	V	E	50

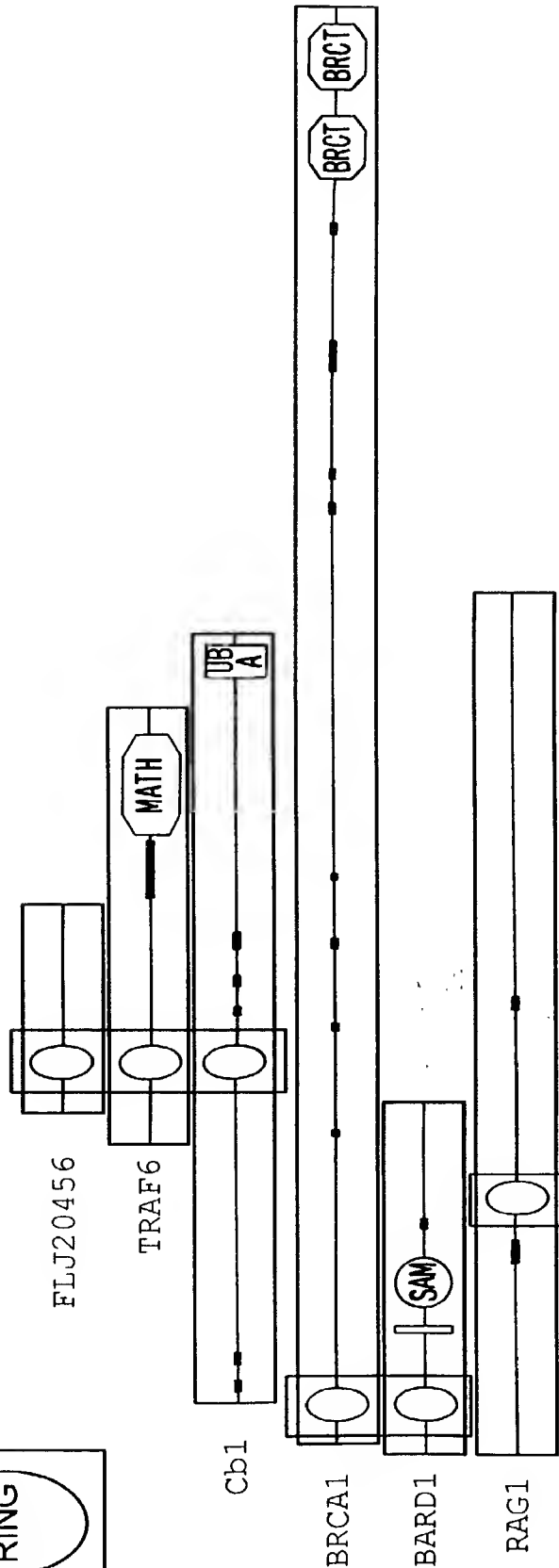
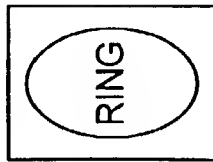


FIG. 8.

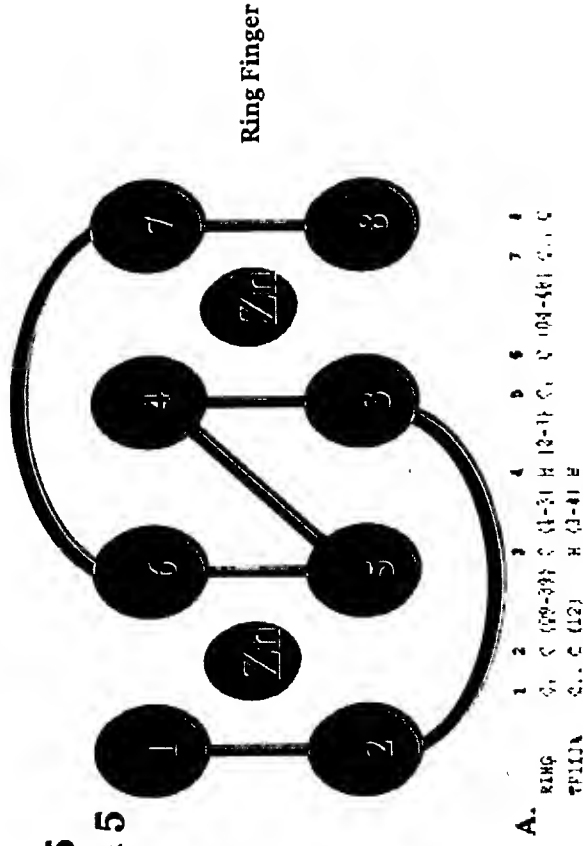
+

RING finger vs. Zinc finger proteins

Figure 9

Ring-HC: C_3HC_4 = Cys in position 5
 Ring H2: $C_3H_2C_3$ = His in position 5

- Ring finger domains have a conserved pattern of Cys and His residues that coordinate two zinc atoms to form a cross-brace structure



- Ring fingers are structurally distinct from zinc fingers

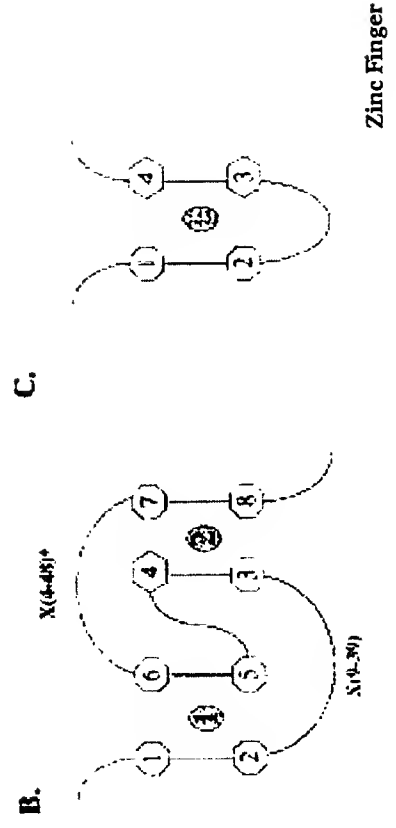


Figure 10A

Ubiquitin Pathway Components

- E1: ubiquitin-activating enzyme, with a major isoform that may work broadly
- E2: ubiquitin-conjugating enzyme, a class of ~14 enzymes, interacts with E3
- E3: ubiquitin ligases, a broad and growing group of activities that promote addition of ubiquitin to specific proteins
- Proteasome-a 26S complex containing a 19S lid and base that mediates ATP- and ubiquitin-chain-dependent binding of substrates and a 20S catalytic core with three known proteolytic activities.

Enzymology of Ubiquitination

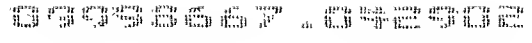
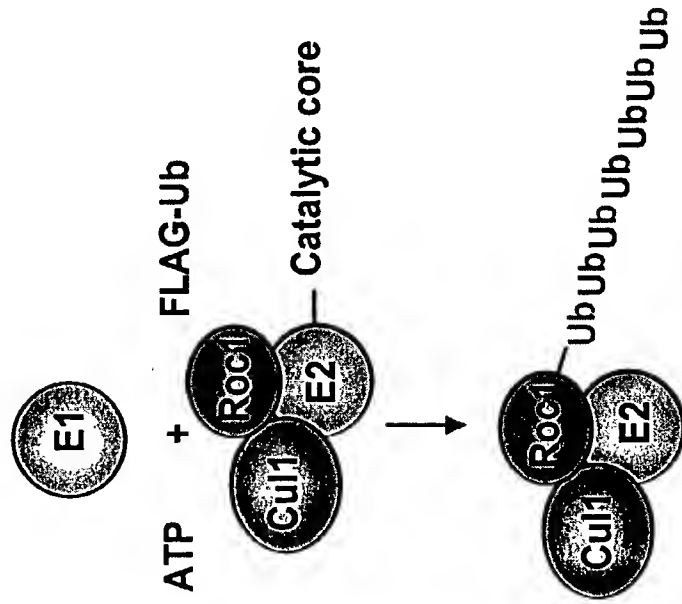


Figure 11A

A Reconstituted, Substrate-independent Assay for Studying Ligase Catalysis



The substrate-independent reaction has the same catalytic properties and requirements for Roc1/Cul1 as the substrate-dependent reaction

Reaction Components

E1:

E2 (UbcH5): GST-fusion (cleaved), E.coli

E3 (Ring/cullin): His-tagged, coexpressed, baculovirus

Ubiquitin: FLAG-tagged, E. coli

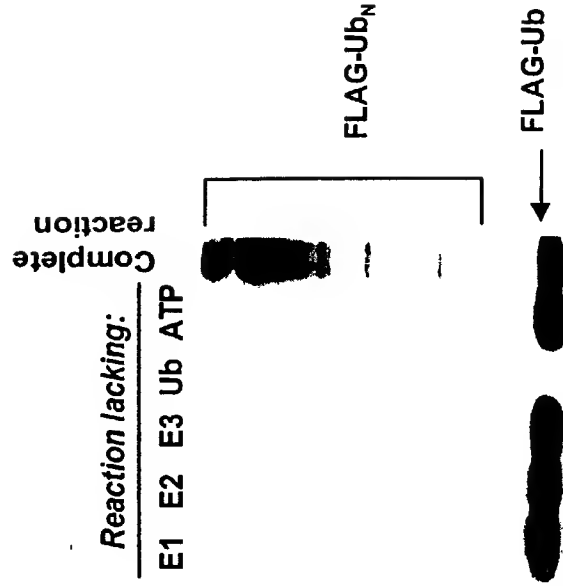
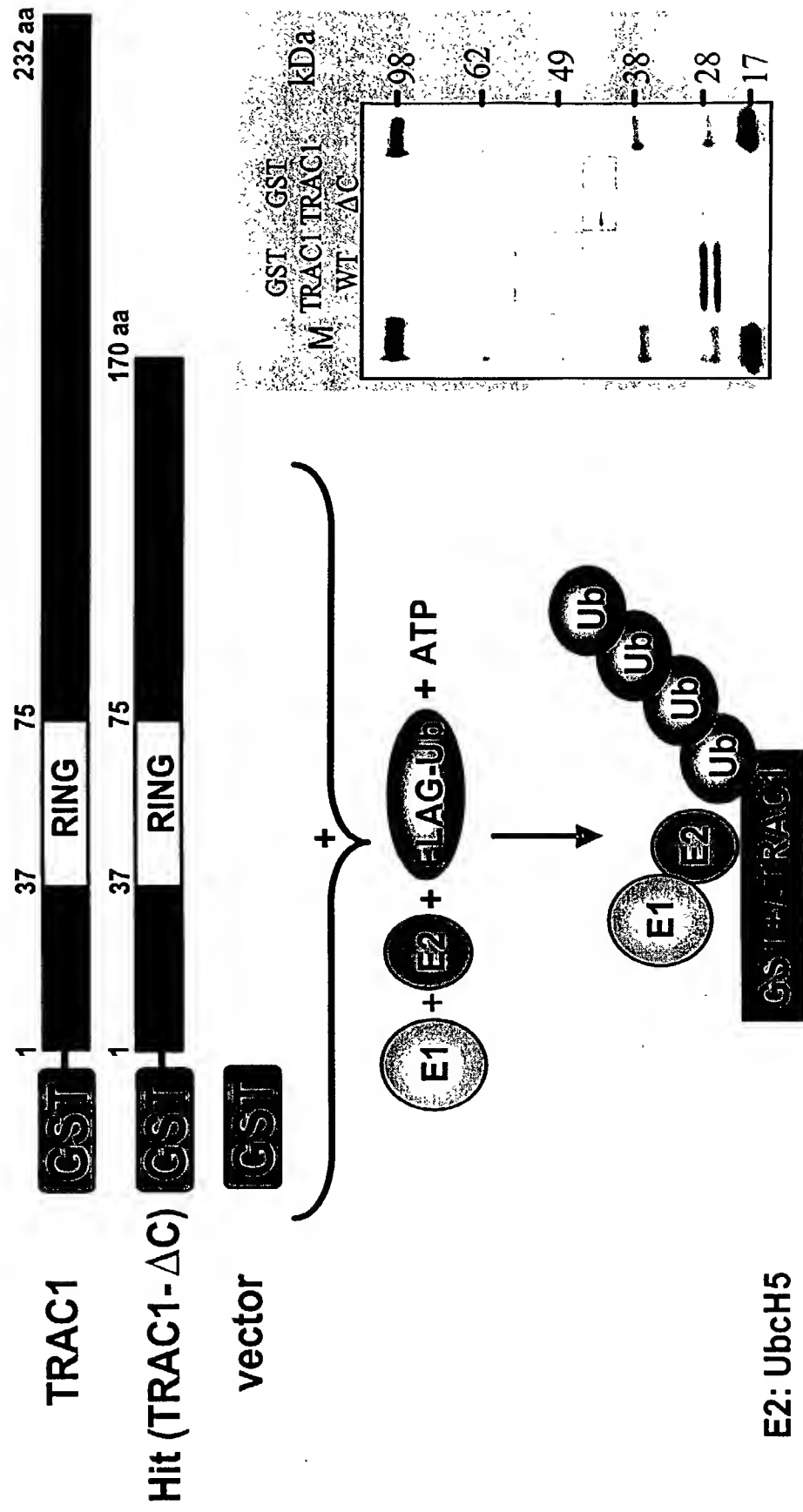


Figure 11B Bacterially-expressed TRAC1 for Ligase Activity Assay



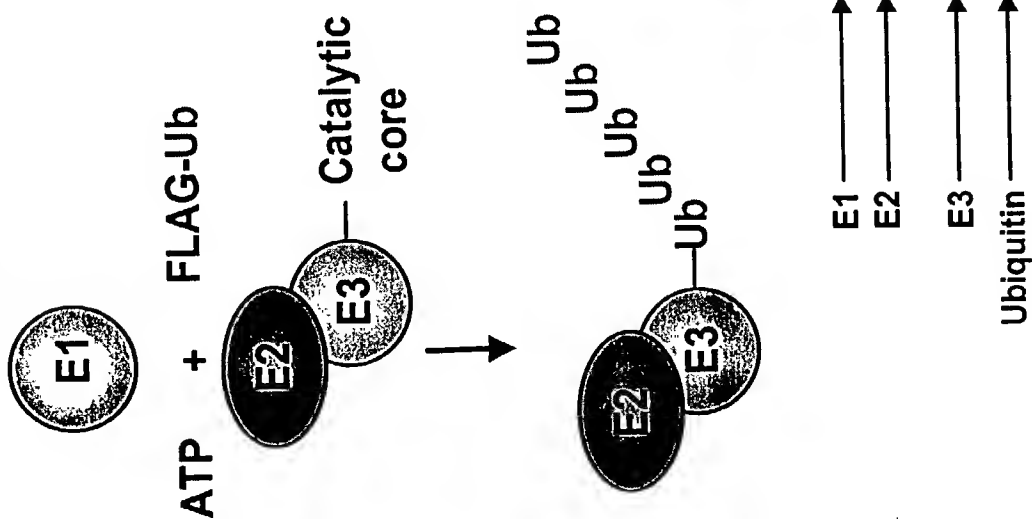
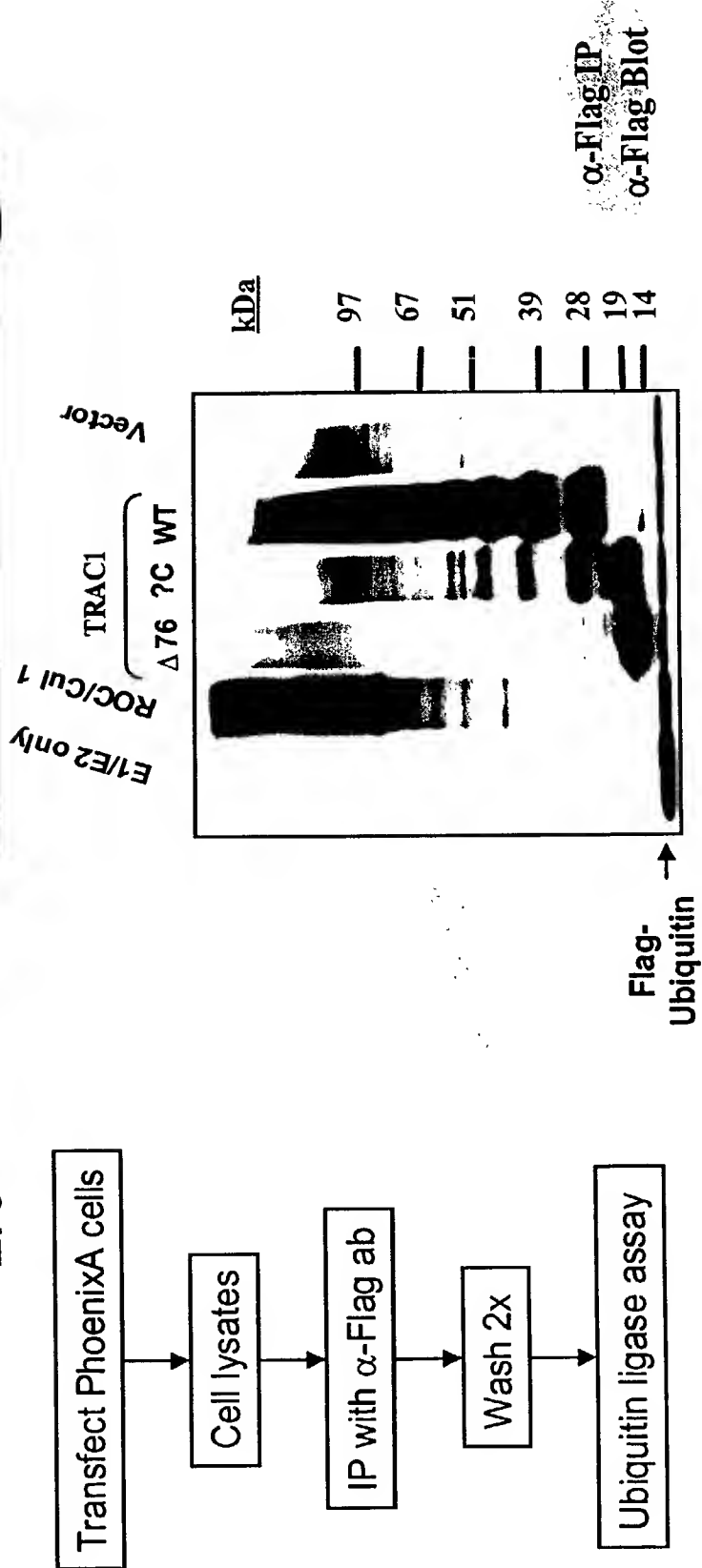
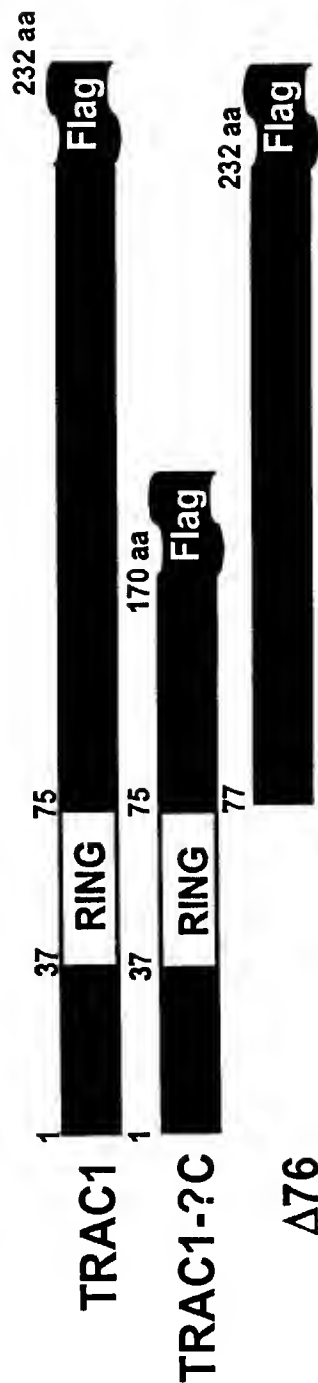
[illegible][illegible]

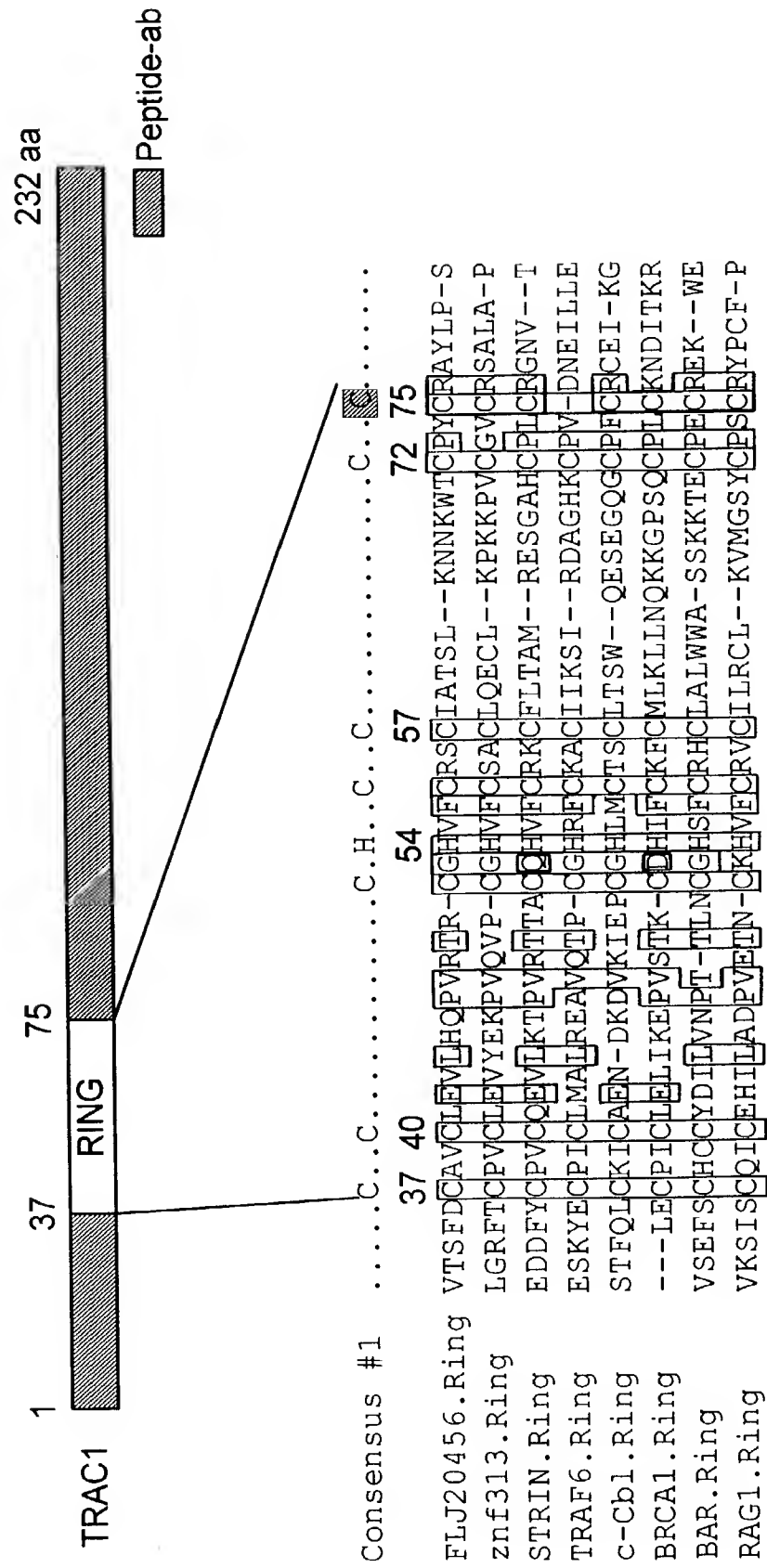
Figure 12B

The Ring Domain is Required for TRAC1 Ligase Activity



+

Point mutations in Conserved Cysteine Residues of the TRAC1 Ring finger Domain



- The following expression plasmids were generated:
 pEFnig/Ring finger point mutants: H54A, C75A, C37, 40A, H54C57A, C72, 75A
 pEFnig/Myristylation site mutant: G2A

FIG. 13A.

+

Figure 13B

Point mutations in Conserved Residues of the TRAC1 Ring-finger Domain Disrupt Ligase Activity

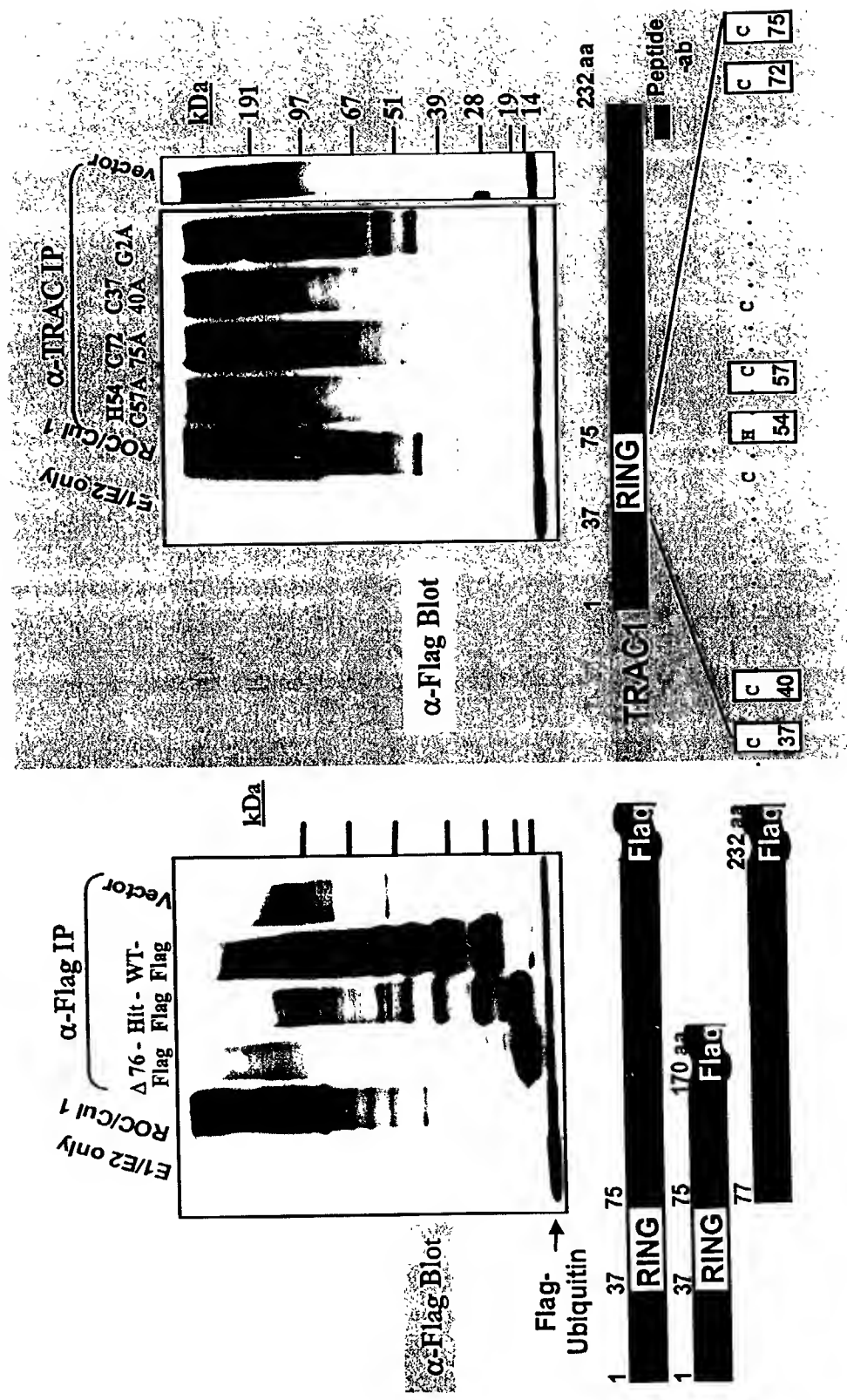
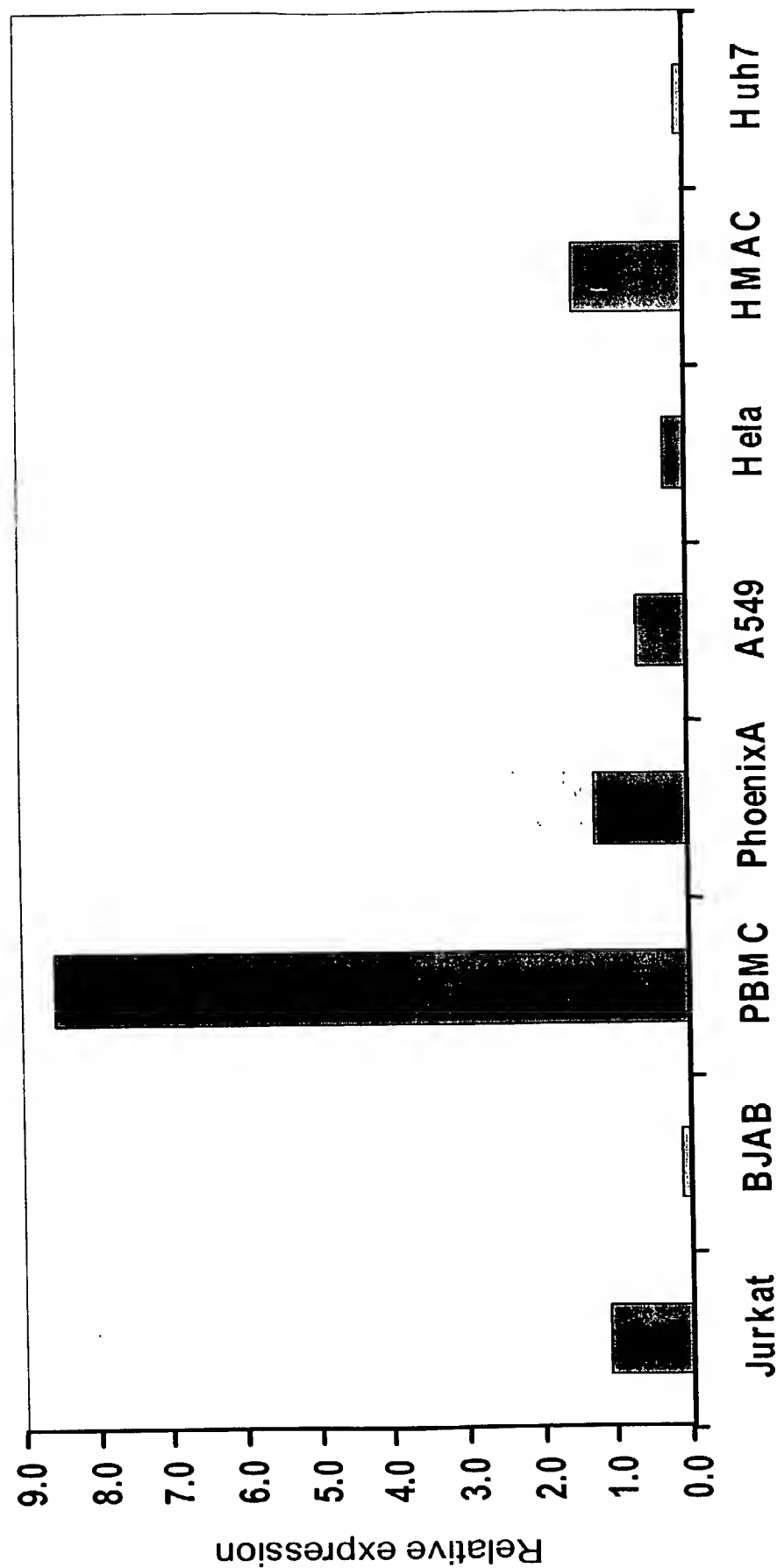


Figure 14

**Expression of TRAC-1 mRNA is ~8 fold
higher in PBMC than in Jurkat cells**

■ TRAC1/HuPO



0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0

Figure 15A

C-terminal Truncated TRAC1 Blocks TCR-induced Ca^{2+} Influx

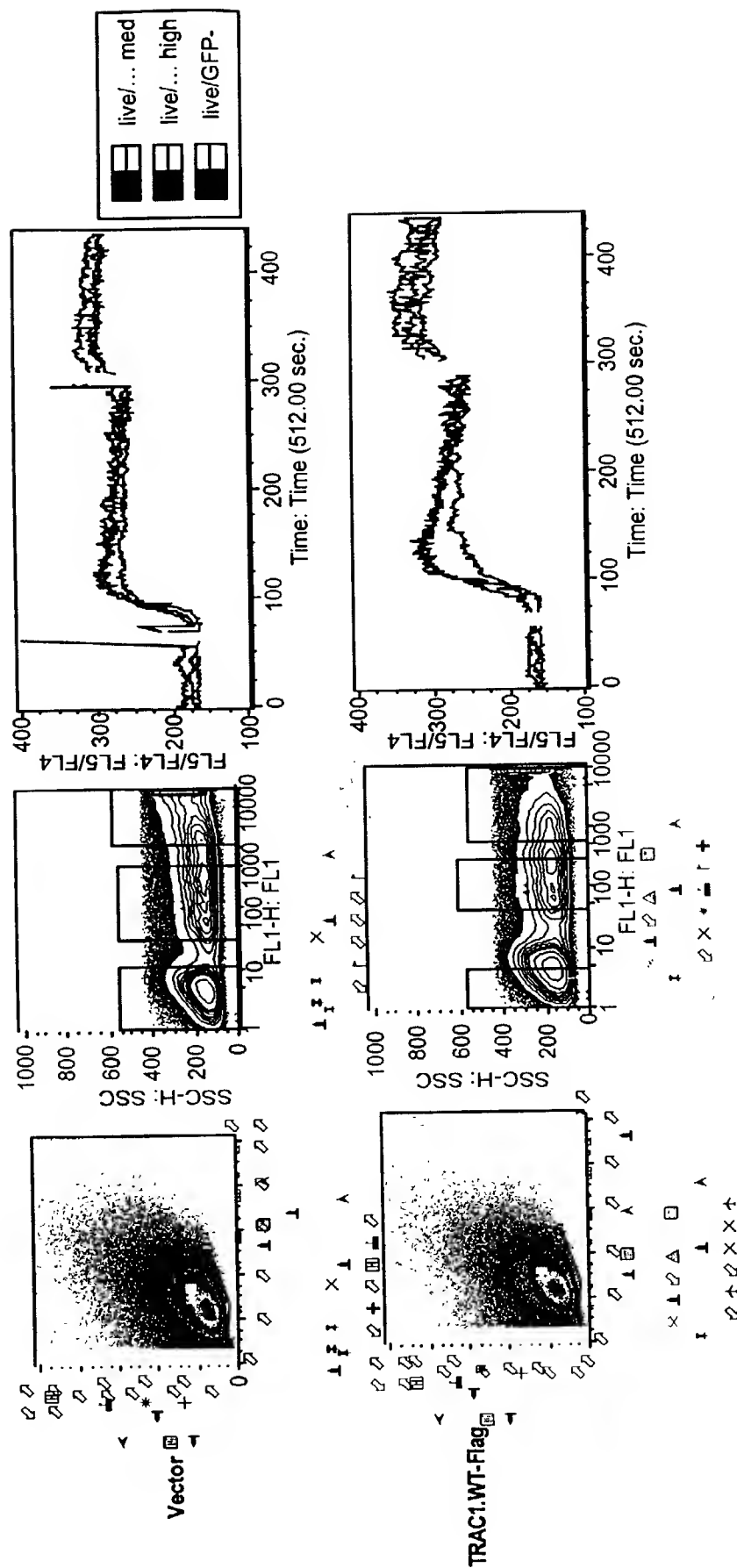


Figure 15B

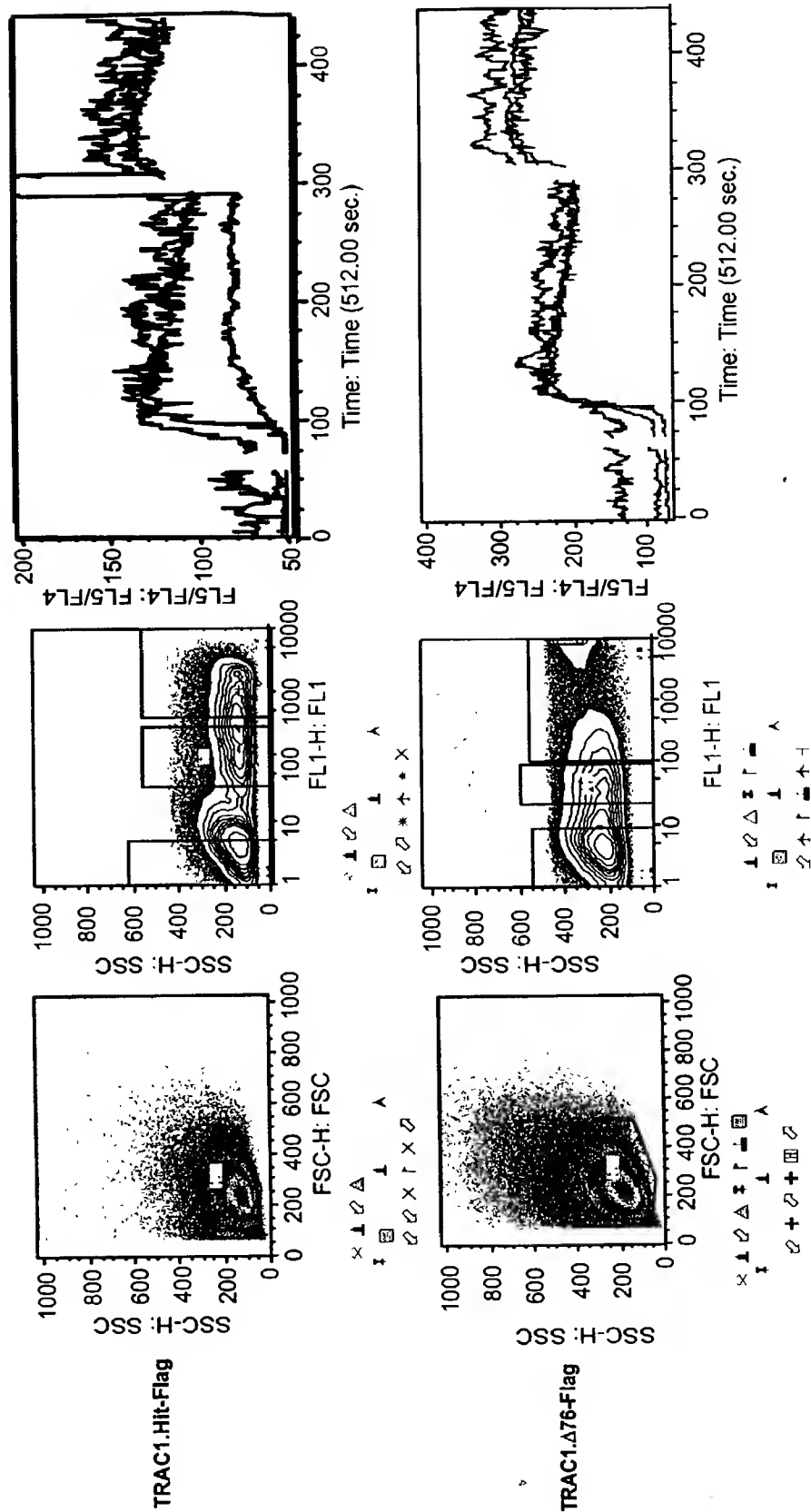


Figure 16

An Intact TRAC1 Ring domain is Required for Inhibition of α -TCR-Induced CD69 Up-regulation

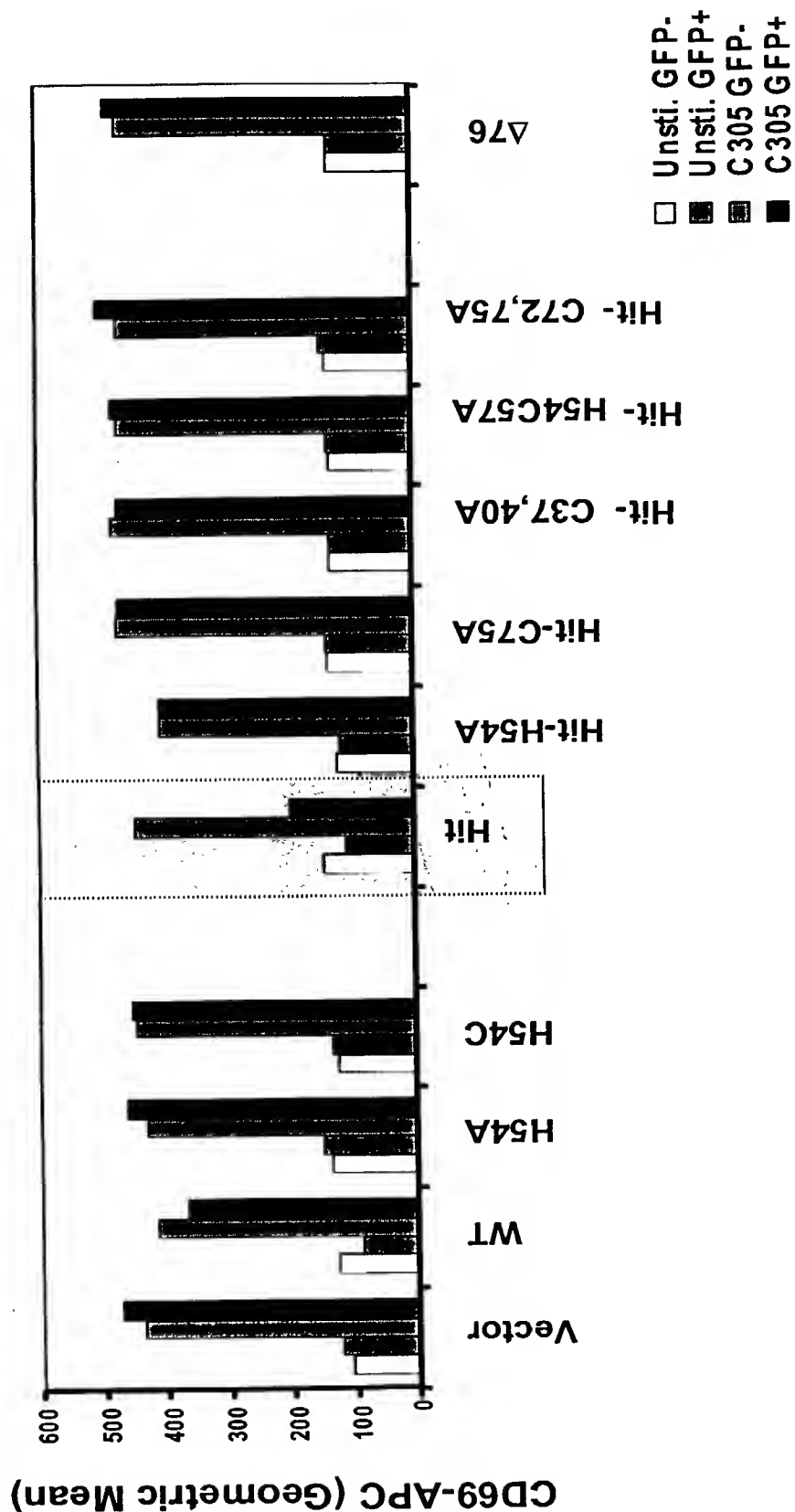


Figure 17

Summary of Functional Effects by Different TRAC-1 constructs

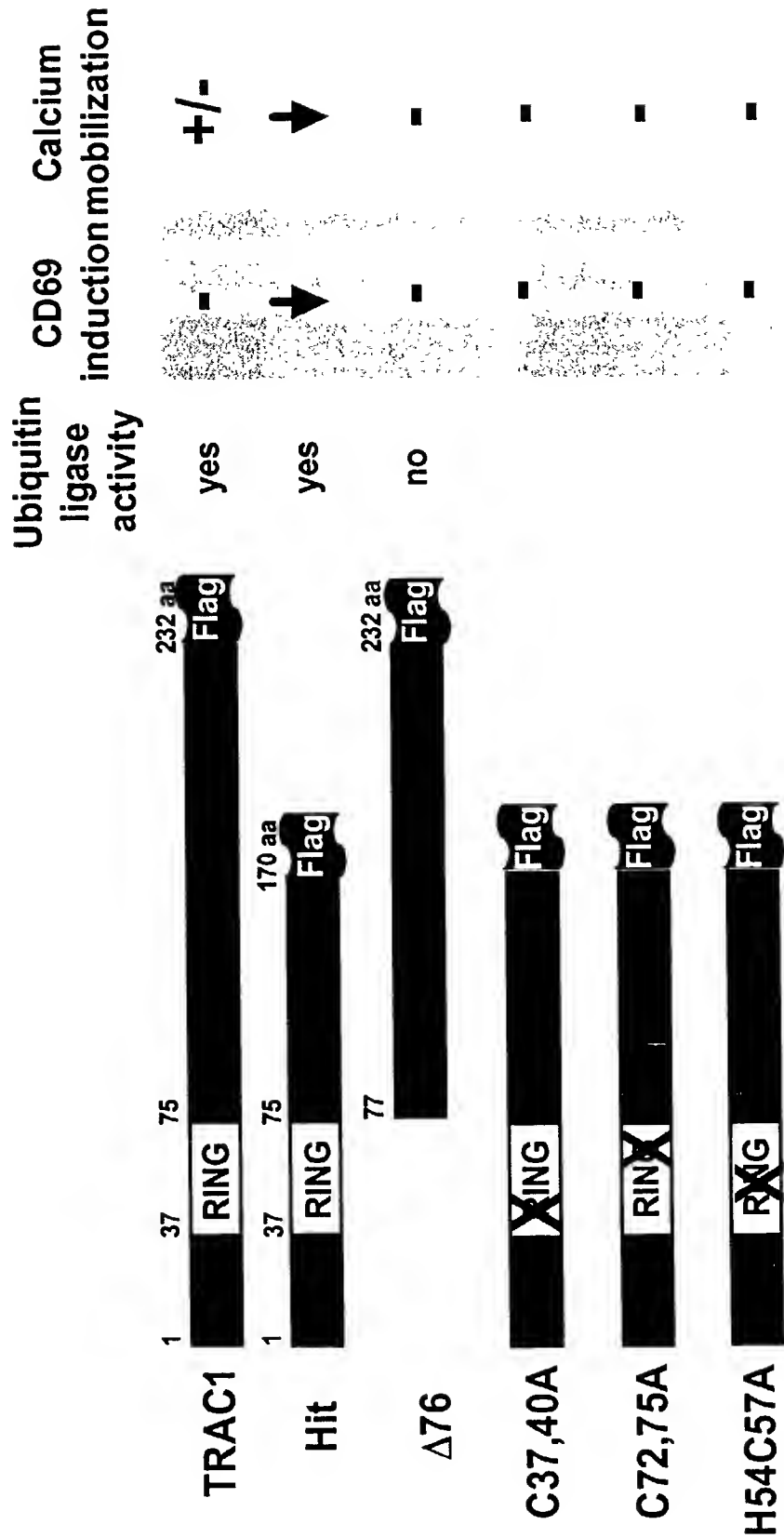
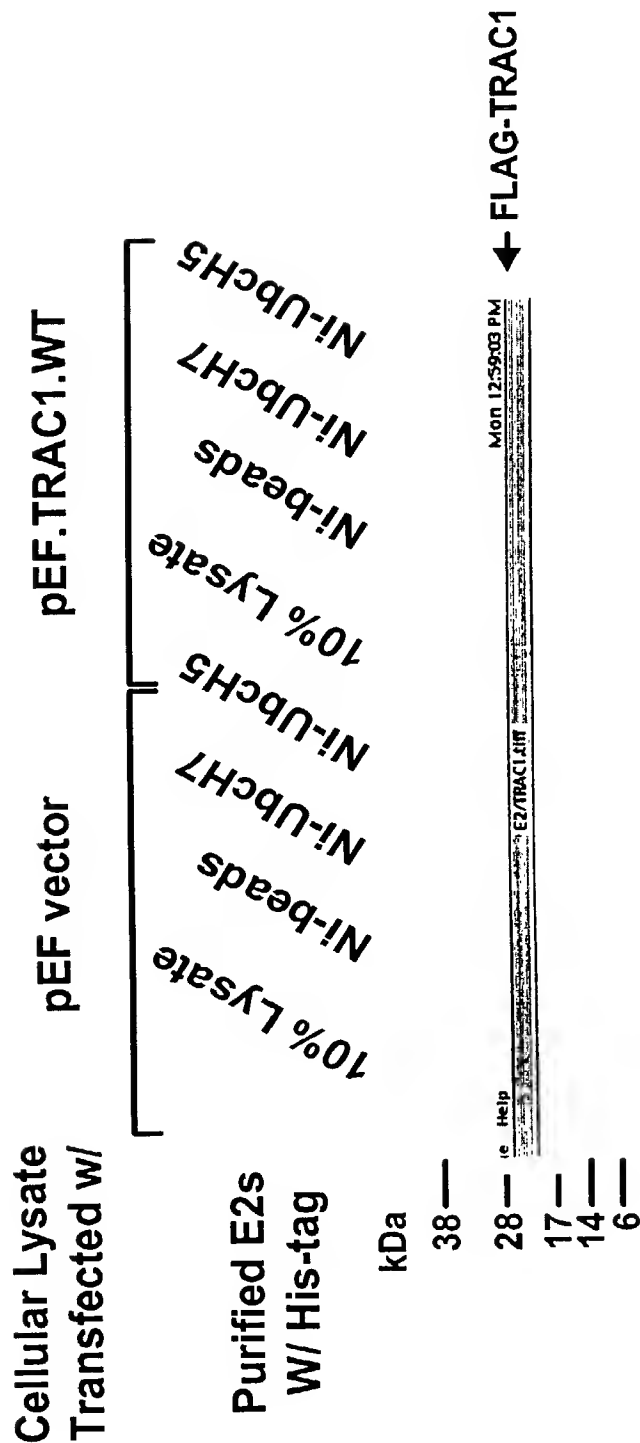


Figure 18

Transiently Transfected TRAC1 Protein Binds to Ubiquitin - Conjugating Enzymes (E2s) UbcH7 and UbcH5 *in vitro*



[illegible]

TRAC1

